

Development of Biologically Modified Anodes for Energy Harvesting Using Microbial Fuel Cells

by James J. Sumner, Rahul Ganguli, and Brad Chmelka

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Development of biologically modified anodes for energy harvesting using microbial fuel cells

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ABSTRACT

Biological fuel cells hold promise as an alternative energy source to batteries for unattended ground sensor applications due to the fact that they can be extremely long lived. This lifetime can be extended over batteries by scavenging fuel from the deployed environment. Microbial fuel cells (MFC) are one class of such sources that produce usable energy from small organic compounds (i.e. sugars, alcohols, organic acids, and biopolymers) which can be easily containerized or scavenged from the environment. The use of microorganisms as the anodic catalysts is what makes these systems unique from other biofuel cell designs. One of the main drawbacks of engineering a sensor system powered by an MFC is that power densities and current flux are extremely low in currently reported systems. The power density is limited by the mass transfer of the fuel source to the catalyst, the metabolism of the microbial catalysts and the electron transfer from the organism to the anode. This presentation will focus on the development of a new style of microbially-modified anodes which will increase power density to a level where a practical power source can be engineered. This is being achieved by developing a three dimensional matrix as an artificial, conductive biofilm. These artificial biofilms will allow the capture of a consortium of microbes designed for efficient metabolism of the available fuel source. Also it will keep the microbes close to the electrode allowing ready access by fuel and providing a low resistance passage of the liberated electrons from fuel oxidation.

Keywords: microbial fuel cell, biofilm, alginate, biological catalysts, waste to energy

1. INTRODUCTION

Microbial fuel cells (MFCs) are biological fuel cells which utilize electrons produced during bacterial metabolism to produce electrical currents.¹ They are typically composed of a cathode and an anode, which are connected externally through a load and internally through an electrolyte and a cation exchange membrane that limits both transport of dissolved oxygen to the anode and movement of microbes to the cathode.¹ Microbes in proximity to the anode anaerobically metabolize organic substrates, liberating protons and electrons.

Electron transport to the anode is either direct, through physical contact of the microbes via cytochromes or putative nanowire structures, or indirect, via a soluble redox active mediator.²⁻⁷ The role of the microbes in a MFC is to produce enzymes that catalyze the oxidation of fuel, which may or may not occur at the anode. Donation of electrons to the anode by microbes in a MFC is advantageous for the organism, especially obligate anaerobes, because it allows them to indirectly utilize the oxygen at the cathode as an electron acceptor while remaining in an anoxic environment. At the cathode, protons combine with dissolved oxygen and electrons that travel from the anode through the external load to form water.⁸

An MFC allows for the complete oxidation of small organic acids, such as lactate, to carbon dioxide and water with organisms from the genera *Shewanella*. The anode compartment is kept anaerobic with a gas tight seal for the electrode lead to pass through and the Nafion membrane which limits oxygen transport from and proton transport to the cathode. The main products of the anode reaction are carbon dioxide, protons and electrons. The cathode is kept aerobic to allow for the protons and electrons to recombine with oxygen to form water.

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1.1 MFC benefits

Biofuel cells can be separated into two classes, enzymatic and microbial. Biofuel cells have been championed for their ability to use non-flammable, non-hazardous fuels and at very high efficiencies. Enzyme based fuel cells use catalytic proteins which have been purified from organisms and immobilized on the electrode surfaces. The goal is to at very high efficiency, generate power from molecules such as sugars, organic acids and alcohols. There are three main challenges in this type of fuel cells: 1) if the enzyme is damaged (from temperature, time or too high of fuel concentration) and is no longer functional it cannot be replaced, 2) a single enzyme can only partially oxidize a fuel, therefore to complete oxidization a relatively simple fuel such as glucose requires multiple enzymes which would all have to be immobilized and functional simultaneously and 3) the power density is reduced as compared to chemical fuel cells.

MFCs are unique and hold some real advantages over enzymatic or even chemical fuel cells under certain circumstances where only low power densities are required. First of all is the regenerating nature of microbes. If an organisms dies or is removed it can be replaced through reproduction of the remaining organisms. Through constant regeneration the density of functional “catalyst” structures on the anode stays relatively constant. Another advantage of microbial systems is that they can consume a whole host of fuels while chemical or enzymatic fuel cells rely on one specific fuel.

Fuels for MFCs are readily available in supplies chains or in the environment including the simple organics used in other systems as well as environmentally available fuels such as crude oil, diesel, chlorinated solvents, industrial, agricultural and domestic wastes including chitin, cellulose, and carbohydrates. Efficiencies of MFCs operating on these complex fuels has been reported to be as high as 65% for consortia and with single organisms being near 50%. The advantages of using microorganisms as your catalysts are pictured in Figure 1.

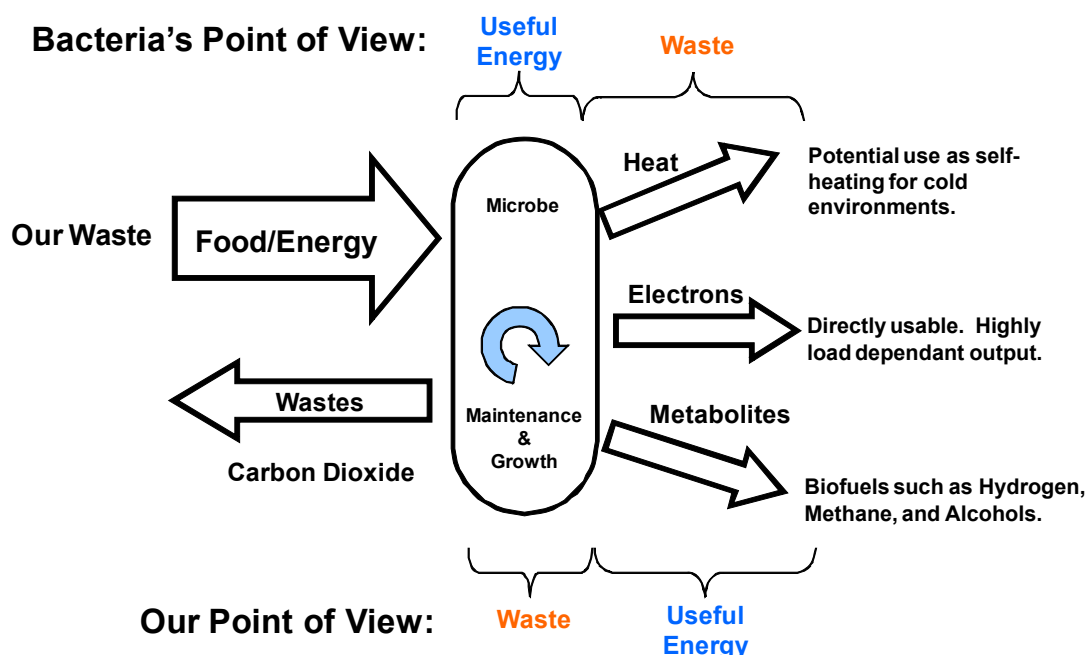


Figure 1. The advantages of utilizing microorganisms as your catalyst are summarized. Things that are considered wastes can be utilized as the organisms energy source and what they deem as waste, such as heat, free electrons and metabolites, can be useful for our applications.

The organisms frequently used in MFCs have many advantages in terms of robustness and availability. These organisms are typically found in locations such as soil, waterways, wastewater, and decomposing organic material. Growth in these environments requires metabolic diversity and results in exposure to stress conditions. As a result, the organisms found in these environments have built in self-preservation mechanisms and are able to survive dramatic temperature

variations, desiccation and starvation. These resistance mechanisms allow the fuel cells to be left unattended for long periods of time at which point they can be revived by providing the appropriate environment. These properties allow for easy isolation of organisms which can be used to create rugged systems with a reasonable storage shelf-life. These characteristics demonstrate that there are many possible applications for MFCs in industry, the laboratory and the field.

1.2 Anode design

Many researchers studying electrogenic bacteria, both in the laboratory and in the field, commonly utilize graphite electrodes because they are inexpensive and these bacteria will readily colonize them. This is consistent with the bacterial use of humates as electron acceptors, as the conductive end planes of graphite can possess many of the same functional groups that are present in humates, including carboxylic acids, alcohols, and quinones. The surface of the graphite electrodes is therefore a very similar to the natural habitat for the bacteria, except that graphite is more conductive allowing the bacteria to more easily dispose of their waste electrons.

In many cases it is thought that bacterial cytochromes are responsible for the final electron transfer to the electrode and that the carboxylic acid termini of self-assembled monolayer (SAM)-modified electrodes can facilitate the transfer through strong hydrogen binding with the peptide bonds of the cytochromes.^{9,10} In trying to clarify the mechanism of bacterial interaction with conductive carbon electron acceptors we can leverage the large body of work that has characterized electron transfer between cytochrome c and acid-terminated SAMs of alkanethiols on gold^{9,11,12,13}. In addition, the extensive work of Whitesides concerning the attachment, and long term viability, of cells on SAMs.¹⁴⁻¹⁸ In particular, it has been observed that bare gold is a poor choice of electron acceptor for both cytochromes and electrogenic bacteria, possibly due to a band gap mismatch. In previous work, we conjectured that we could enhance the electronic coupling between gold electrodes and electrogenic bacteria by forming a carboxylic acid terminated alkanethiol SAM on the electrode surface. In this report, we describe marked increases in current production for carboxylic acid terminated alkanethiol SAMs over both bare gold and methyl terminated SAMs. Figure 2 displays representative data of how microorganisms can electronically couple with bulk electrodes surfaces.

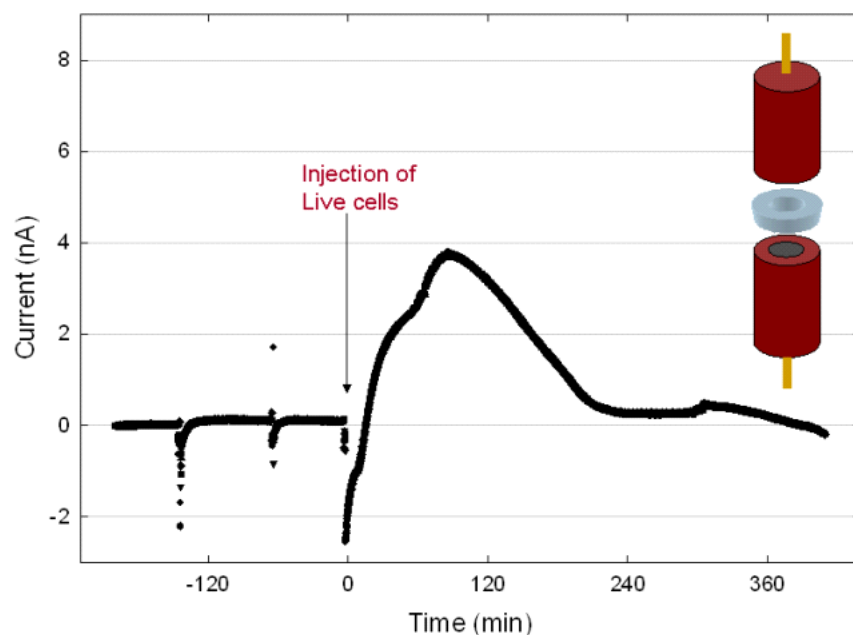


Figure 2. Plot of representative data of how microorganisms can electronically couple with bulk electrodes surfaces. The negative spikes in current come from the ions added with the killed cells but current generation only occurred with live cell injections. The schematic in the upper right corner is of the simple electrode configuration used to collect this data.

Conventional MFCs have the disadvantage that typical experiments take on the order of weeks to months for the bacteria to colonize the electrode, produce power, and exhaust the fuel. In order to reduce the time required for these experiments we developed a microliter-scale MFC and injected a sufficient number of bacteria at the start of the experiment to enable relatively instantaneous colonization of the electrode. This allows us to “turn-on” the fuel cell in seconds to minutes instead of days and complete a full run in hours instead of weeks. Our membraneless MFC, with a total volume of approximately 10 μL , is constructed from closely spaced standard electrochemical electrodes. Current production is seen almost immediately upon the addition of a suspension of cells in minimal medium. Typical MFCs utilize two chambers with the anodic and cathodic compartments separated by a proton exchange membrane. However single-chamber systems have been demonstrated and are simpler to construct at the 10 μL scale. We describe our use of these microliter-scale MFC assemblies to rapidly explore electronic coupling between electrogenic bacteria and various electrode materials including glassy carbon, gold and SAM modified gold.¹⁹

While this technique was useful to rapidly screen organisms, fuels and electrode materials it did not allow for the formation of robust, electroactive biofilms. This paper describes the application of an artificial biofilm that allows for rapid assembly that is also robust and conductive.

2. MATERIALS AND METHODS

Artificial, conductive biofilms were prepared of both yeast and bacteria from the genera *Shewanella*. The procedures varied slightly due to growth requirements and yeasts requirement for an electron mediator. All electrochemical data was collected via linear sweep voltammetry employed by a CH Instruments electrochemical workstation model 660a or a National Instruments DAQ with in-house written software using LabView.

2.1 Yeast

Yeast biofilms were all prepared using a minimal media referred to as M9 with glucose added. M9 consists of 3g KH_2PO_4 , 6g Na_2HPO_4 , 5g NaCl , 1 mL of 1 M MgSO_4 and dilute to 1 liter of deionized water. Glucose was held at a concentration of 4%. The yeast was cultured in the presence of a carbon felt electrode (~1cm X4cm) with a titanium wire as the electrical lead. 7 micron carbon fiber was cleaved to lengths of less than 1 cm and further fractured in a homogenizer. Alginate serves as the binding matrix and it is prepared from the sodium form in a solution of the M(with varying dopings of the fractured carbon fiber. The alginate hydrogel matrix is set by soaking in an aqueous solution of calcium chloride. This artificial biofilm can then be stained with a mediator by soaking in a solution of the mediator (typically methylene blue) prepared in the M9 solution.²⁰

2.2 Shewanella

The only main differences between the preparation of *Shewanella* and yeast films are with the media and mediators. The media for *Shewanella* can be either LB broth, fresh M9 with lactate added as the fuel or spent M9 from yeast cultures. The immobilization is performed as described earlier. Also mediators were not added to these films as this bacteria can transfer electrons without mediators present.

3. RESULTS AND DISCUSSION

There were two main goals for the presentation here. One was preparation and electrochemical characterization of the artificial biofilms and the other was an example of utilizing these biofilms.

As for the preparation, figure 3 shows a summary of the results of the yeast biofilm preparation. The image on the right is a scanning electron micrograph of the prepared yeast biofilm using 4% by weight carbon fiber deposited onto an electrode. The carbon fibers are clearly seen however, it is required to zoom in on the image to see the yeast cells. These are displayed in the images in the lower left of the figure. The upper left is a plot of the power produced by these biofilms vs. the current. These power curves show that doping the alginate hydrogel dramatically increases power production but overdoping starts to hinder power this is possibly due to physically blocking passages in the hydrogel that allow for food to reach the cells and waste products to escape the biofilm.

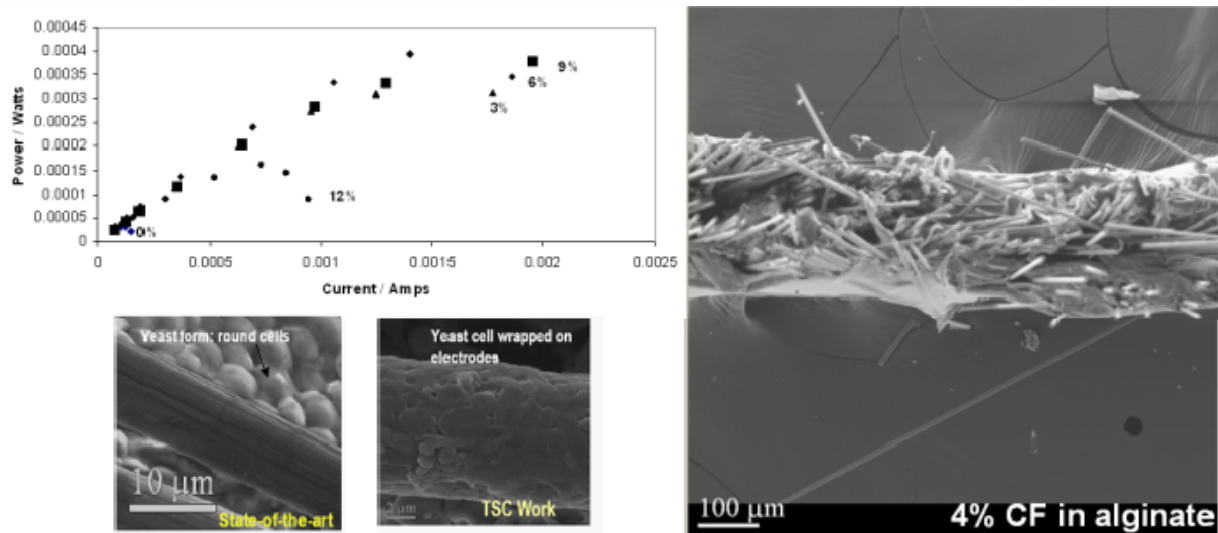


Figure 3. The image on the right is an SEM of the carbon fiber doped, alginate-based artificial biofilm. The images in the lower left are magnifications of the carbon fibers to show the yeast cells and the upper left plots power curves of different doping of the artificial biofilms by weight percent. Over doping the biofilms does hinder performance.

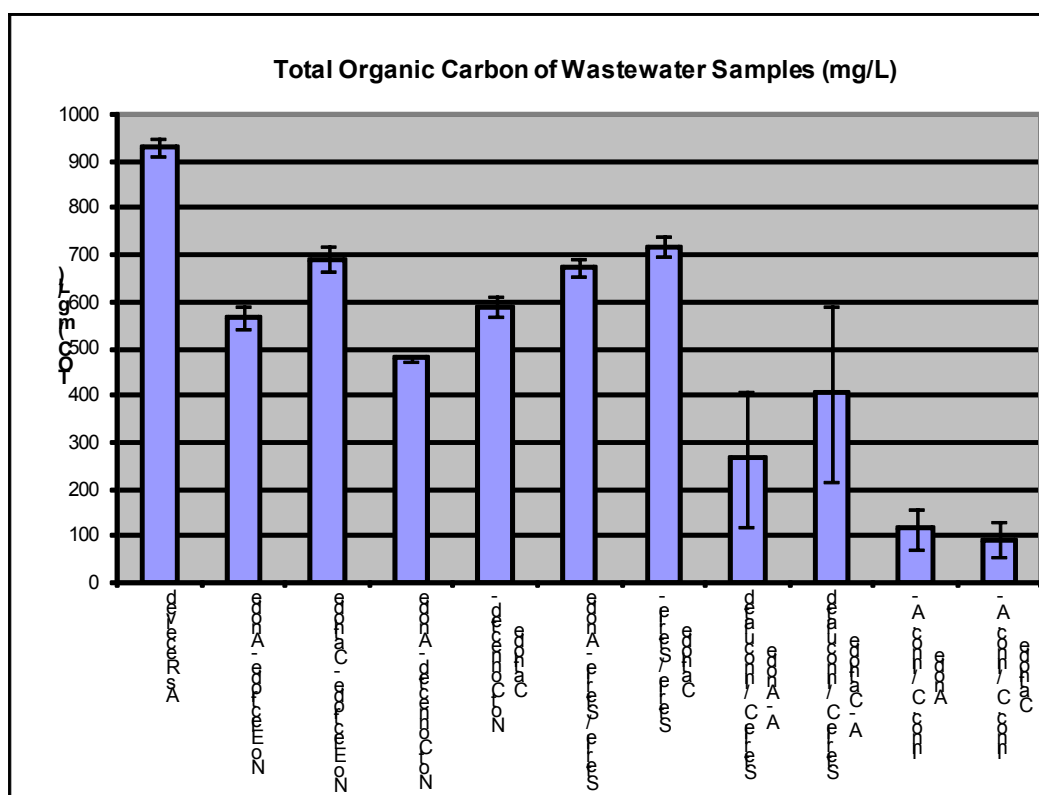


Figure 4. This graph shows the enhancement of removal of total organic carbon from sample wastewater as opposed to continuing a typical anaerobic digestion. The controls also show the effect of the graphite electrode and sterilization of the wastewater samples via autoclave.

Finally, figure 4 is a graph demonstrating one of the possible applications on the artificial, conductive biofilms. This is an experiment demonstrating how MFCs can be utilized to enhance the reduction of total organic carbon (TOC) of wastewater samples. This is a necessary step in wastewater treatment and in normal practice this is a aerobic process which is extremely energy intensive. In an attempt to remove the need for mixing and aerating the sample, microbial fuel cells were inoculated with wastewater samples and held to strict controls to determine performance. Each sample from left to right was selected to show control over the system. The first shows that the wastewater as received was above 900 mg/L and if allowed to continue to digest in a MFC housing without electrodes present, TOC was only reduced to ~600 mg/L. Since the electrodes are composed of graphite there is further reduction of TOC even if the electrode are not connected or if the wastewater was sterilized via autoclave simply due to organics being absorbed by the graphite. With the presence of an inoculated anode and sterile cathode enhanced removal of TOC but increased error in the measurements. The last two data points show inoculated anode and cathode reducing the TOC to ~ 100 mg / L.

4. CONCLUSIONS

Microbial fuel cells are novel bioelectrochemical devices capable of converting organic waste and environmentally available fuels into electricity and commodity chemicals. While the power density of these devices is low there are several niche applications where MFCs could prove viable including: power for unattended ground sensors, waste mitigation, battery trickle chargers, self-powered sensing and enhanced biofuel production. While there are many technical barriers to each of these applications there has been great progress in overcoming these over the past several years. Investment is still required in several areas in order to achieve these goals. These research areas include: Inexpensive electrodes, catalysts, scale-up designs, field tests, electron transfer mechanisms, fermentation control, designed consortia, and engineering of organisms and populations for optimal operation. While there are currently operating demonstration systems for many different applications all over the world the community is still striving for industrial scale applications. However this leaves a unique opportunity. Some small scale applications could be demonstrated on a much shorter time scale than industrial systems and could enable the technology to be applied more broadly in the future. One example application is waste mitigation at forward operating bases, increasing situational awareness and decreasing logistics required for waste management. There are also exciting possibilities of using MFCs for unattended ground sensors which could eventually be powered by environmentally available fuels thus eliminating the need to replace batteries or whole sensors in dangerous areas.

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